Because one of the PI3Ki resistant lines in the current paper was ER negative, additional mechanisms likely underlie persistent CDK4 activation in ER-negative PI3Ki-resistant breast cancer lines. Indeed, a comprehensive elucidation of the mechanisms underlying persistent cyclin D1 expression and CDK4 activity in the presence of PI3Ki and the downstream events mediating cell survival and proliferation may provide a "suite" of targets warranting exploration in combination with PI3Ki in the future. In the absence of a detailed understanding of the underlying mechanisms, the most attractive clinical option is to explore the combination of PI3K and CDK4/6 inhibitors (Figure 1). Direct targeting of CDK4/6 or critical downstream nodes has the potential to bypass the multiple resistance mechanisms that integrate at cyclin D1 expression and CDK4 activation.

The therapeutic implications of the current study are promising and warrant exploration in clinical trials. First, the combination of PI3K and CDK4/6 inhibitors may be synergistic in different contexts and genomic backgrounds. Second, the addition of CDK4/6 inhibitors to xenograft models that progressed on PI3Ki effectively inhibited tumor growth, suggesting that CDK4/6 inhibitors can prevent the emergence as well as overcome resistance to PI3Ki. Third, persistent pRB represents a potential biomarker to identify patients in which addition of CDK4/6 inhibitors to ongoing PI3Ki treatment may be beneficial. Fourth, in preclinical studies, the combination of PI3K and CDK4/6 inhibitors appears to be well tolerated. Interestingly, clinical trials are underway to test the efficacy of CDK4/6 inhibitors together with rapalogs and aromatase inhibitors (https://clinicaltrials.gov) to determine whether the combination of other PI3K pathway and CDK4/6 inhibitors may benefit patients who have relapsed on prior hormone or PI3K targeted therapies.

In conclusion, this study adds to the growing number of rational combinations with PI3Ki that could fulfill the promise of targeting the PI3K pathway in the clinic. Nevertheless, it remains critical to identify and refine biomarkers that will allow assignment to combination treatments that would lead to the most efficacious response and to continue to identify novel drug combinations that would be well tolerated in patients.

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RhoA Mutations Identified in Diffuse Gastric Cancer

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http://dx.doi.org/10.1016/j.ccr.2014.06.022

The diffuse-type histologic variant of gastric cancer is characterized by highly invasive growth patterns and lack of cellular cohesion. Two recent studies have identified highly recurrent mutations of the gene encoding the small GTPase RhoA and suggest that RhoA activity may have a tumor suppressive role in this disease.

Gastric cancer is the third leading cause of cancer mortality worldwide and, when diagnosed, carries a dismal prognosis. Although gastric carcinoma has marked heterogeneity, the two most salient subtypes are intestinal gastric cancer (IGC) and diffuse gastric cancer (DGC). The dichotomization of IGC and DGC emerged following recognition of their distinct histopathologic growth patterns. IGC is the more common variant and typically emerges following *Helicobater pylori* infection, which leads to atrophic gastritis, intestinal metaplasia, dysplasia, and finally carcinoma. Similar to most adenocarcinomas, IGC typically shows cohesive groups of tumor cells with a glandular architecture. DGC, by contrast, owes its name to its characteristic lack of cellular cohesion, invasion throughout the stroma, and poor cellular differentiation (often with a signet-ring cell morphology). Clinically, DGC's propensity for invasion translates

into early metastasis and poor survival. Moreover, unlike in IGC where tumors with *ERBB2*-amplification are treated with trastuzumab, we lack effective targeted therapies for DGC.

In addition to their histopathologic features, there are underlying biologic and genomic distinctions between DGC and IGC. A key finding shaping our understanding of DGC was the discovery that families with a hereditary form of DGC carried a mutation in *CDH1*, which encodes E-cadherin (Guilford et al., 1998). Beyond hereditary DGC, the vastly more common sporadic form of DGC has also been associated with E-cadherin loss, either through somatic mutation or promoter hypermethylation. Recently, a pair of studies published in *Nature Genetics* expands our understanding of DGC by describing novel recurrent mutations of *RHOA*, encoding the small GTPase RhoA, in 14.3%–25.3% of DGC patients (Kakiuchi et al., 2014; Wang et al., 2014).

Wang et al. (2014) performed whole-genome sequencing, DNA copy number, gene expression, and DNA methylation analyses of 100 tumor and nontumor paired samples, spanning both IGC and DGC. Their analysis revealed frequent mutations in *TP53* in both subtypes, *ARID1A* in EBV-related or microsatellite instability-related cancers, and *CDH1* in DGC. *RHOA* mutation was identified recurrently within DGC and, following sequencing in a larger DGC cohort, was found in 14 of 98 DGC patients (14.3%). Kakiuchi et al. (2014) initially performed whole-exome sequencing within 30 DGCs and focused sequencing in another 57 cases, finding *RHOA* mutations in 22 of 87 (25.3%) cases.

These findings and similar results emerging from The Cancer Genome Atlas study of gastric adenocarcinoma (unpublished data) implicate *RHOA* as a novel candidate driver of DGC. RhoA is a member of the Rho family of small GTPases-Ras-like proteins that act as an intermediary between cell surface receptors and different intracellular signaling proteins. Similar to other GTPases, RhoA cycles between an inactive, GDP-bound configuration and an active GTP-bound configuration that interacts with downstream effectors, such as ROCK, that impact the structure and dynamics of the actin cytoskeleton, cell migration, cytokinesis, and the cell cycle. RhoA overexpression has been observed in various cancers, and RhoA activity has been implicated in tumorigenesis and tumor cell invasion (Karlsson et al., 2009).

Given the characteristic invasive growth patterns that are a hallmark of DGC, mutations in *RHOA* could be predicted to lead to constitutive activation of RhoA, enhancing activity of downstream mediators and increasing cellular invasion. Among the mutations in *RHOA* identified by these two studies, one led to a truncated protein, while the others were missense events with dramatic clustering in the amino terminal portion of the protein. However, the specific *RHOA* mutations identified in DGC were not at sites analogous to oncogenic mutations in RAS-family GTPases that cause RAS to become locked into its active-GTP bound state. These *RHOA* mutations were noted in hotspot sites, including Y42C, G17E, R5Q/W, and L57V. The most common alteration, Y42C, seen in 14 cases, lies in the effector-binding region of RhoA. Although not previously identified in cancer, the Y42C substitution in RhoA had been evaluated in earlier biochemical studies, which revealed attenuated activation of protein kinase N (Sahai et al., 1998). RhoA-Y42 notably corresponds to Y40 on HRas, where mutations selectively reduce HRas activation of RAF, but not other RAS effectors (Joneson et al., 1996), suggesting that the Y42 *RHOA* mutation may similarly modulate RhoA activity. Intriguingly, G17E mutations of *RHOA* were identified in five patients. Recent genomic sequencing studies in T cell neoplasms identified highly recurrent *RHOA* G17V mutations and demonstrated functionally that these mutants fail to bind GTP and act in a dominant-negative fashion to inhibit RhoA GTP loading (Palomero et al., 2014; Sakata-Yanagimoto et al., 2014; Yoo et al., 2014).

To functionally interrogate these novel *RHOA* mutations found in DGC, Kakiuchi et al. (2014) studied several cancer cell lines harboring *RHOA* mutations: the OE19 cell line (adenocarcinoma of the gastric cardia), the breast cancer cell line BT474, and the colorectal cancer line SW948. They showed that small interfering RNA (siRNA)-mediated silencing of *RHOA* significantly impairs in vitro proliferation in these mutant cell lines but does not similarly impact gastric cancer cell lines with wild-type *RHOA*. Furthermore, they demonstrated that reintroduction of the codon 17 or 42 *RHOA* mutants, but not reintroduction of wild-type *RHOA* rescued cell proliferation effects of *RHOA* siRNA, suggesting tumor-promoting activity for these *RHOA* mutants.

The results from Wang et al. (2014) provide additional insights into the potential role of mutant *RHOA*. Using a Rho binding domain assay to immunoprecipitate RhoA-GTP, the authors showed that both the Y42C and L57V mutants significantly attenuate the GTP-associated form compared to wild-type protein, indicating a potential defect in RhoA activation with these mutants. They further utilized primary mouse intestinal organoids to study the impact of *RHOA* mutants Y42C and L57V upon anoikis (cell death induced when anchorage-dependent cells detach from the surrounding extracellular matrix). Inhibition of anoikis may represent a key requirement for DGC, because loss of E-cadherin leading to reduction in cellular adhesion has been shown to result in acute cell death via anoikis (Kantak and Kramer, 1998). With dissociation of the mouse intestinal organoids, the introduction of Y42C or L57V *RHOA* mutants enhanced organoid reformation. While treatment with ROCK inhibitor Y-27632 also enhanced colony growth, wild-type *RHOA* induction reduced the colony forming efficiency.

Through comprehensive genomic characterization, these studies demonstrate that, along with *CDH1* mutations, *RHOA* mutations are quite common in DGC but not in other variants of gastric cancer. Intriguingly, these results suggest a model whereby wild-type RhoA activity has a tumor suppressive role in the pathophysiology of DGC and that *RHOA* mutations inhibit this tumor suppressive function, suggesting these mutants are not merely loss of function, but may repress RhoA activity. It remains to be clarified, however, whether *RHOA* mutations merely attenuate physiologic RhoA activity or, alternatively, if these mutations result in a gain of function. Given the pressing need for new therapeutic targets for DGC, further research will be required to determine if the activity of these novel *RHOA* mutants and the deleterious role of RhoA activity in this disease can be exploited as a therapeutic vulnerability.

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Ibrutinib Treatment of CLL: The Cancer Fights Back

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Ibrutinib is a potent inhibitor of Bruton's tyrosine kinase (BTK). Studies published in the *New England Journal of Medicine* report that patients with chronic lymphocytic leukemia (CLL) have durable responses to ibrutinib, but they also describe the advent of bypass mutations that result in ibrutinib resistance and progressive disease.

Signaling through the B cell receptor (BCR) can promote tumor cell survival in B cell malignancies, including chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and the activated B cell-like (ABC) subtype of diffuse large B cell lymphoma (DLBCL). The BCR consists of immunoglobulin heavy (IgH) and light (IgL) chains coupled to a CD79A-CD79B heterodimer that transduces signals by engaging downstream nonreceptor kinases, including Bruton's tyrosine kinase (BTK) (Young and Staudt, 2013). These kinases offer a wealth of therapeutic targets, and drugs targeting SYK, BTK, and phosphatidylinositol 3-kinase (PI3K) are in clinical trials to evaluate their efficacy against a variety of human lymphomas.

Ibrutinib (PCI-32765, Imbruvica) is an irreversible inhibitor of BTK that works by forming a covalent bond with cysteine 481 (C481) in the BTK active site, rendering the drug potent and highly selective, thereby limiting side effects. Several clinical trials are now evaluating ibrutinib in human lymphomas, and the drug has been granted breakthrough status by the US Food and Drug Administration for the treatment of refractory MCL and high-risk CLL. Because activating mutations in BTK have not been observed in these lymphomas, it is likely that upstream signaling from the BCR is the culprit.

BCR expression is obligatory in normal B cells and most malignant B cells. In CLL, analysis of the antigen recognition portion of the BCR revealed preferential usage of a small subset of Ig variable gene segments, suggesting that the BCRs may react with an antigen. In support of this notion, different CLL and MCL patients can have "stereotypic" BCRs with virtually identical antigen recognition sites (Agathangelidis et al., 2012). The first direct evidence for BCR-dependent survival signaling was obtained in ABC DLBCL (Davis et al., 2010). RNA interference screening revealed that BCR components and downstream signaling effectors (SYK, BTK, and PLCγ2) are required for ABC DLBCL cell survival. Microscopy revealed BCR clusters on the surface of ABC DLBCL cells that are similar to those induced by antigen engagement of the BCR in normal B cells. Recurrent gain-of-function mutations in *CD79A* and *CD79B* augment BCR signaling in a subset of ABC DLBCL cases, providing genetic evidence that the BCR pathway is important in the pathogenesis of this lymphoma subtype. The "chronic active" form of BCR signaling in ABC DLBCL is sensitive to ibrutinib and therefore may be mechanistically similar to BCR signaling in CLL and MCL (Figure 1).

Three reports in the *New England Journal of Medicine* examined ibrutinib treatment in CLL patients. The first study evaluated ibrutinib monotherapy in patients with relapsed and high-risk CLL versus ofatumumab, an anti-CD20 antibody that is the current standard therapy for these patients. Ibrutinib produced a 70% response rate compared with only 21% for ofatumumab, and ibrutinib was also superior to ofatumumab with respect to progression-free and overall survival (Byrd et al., 2014).